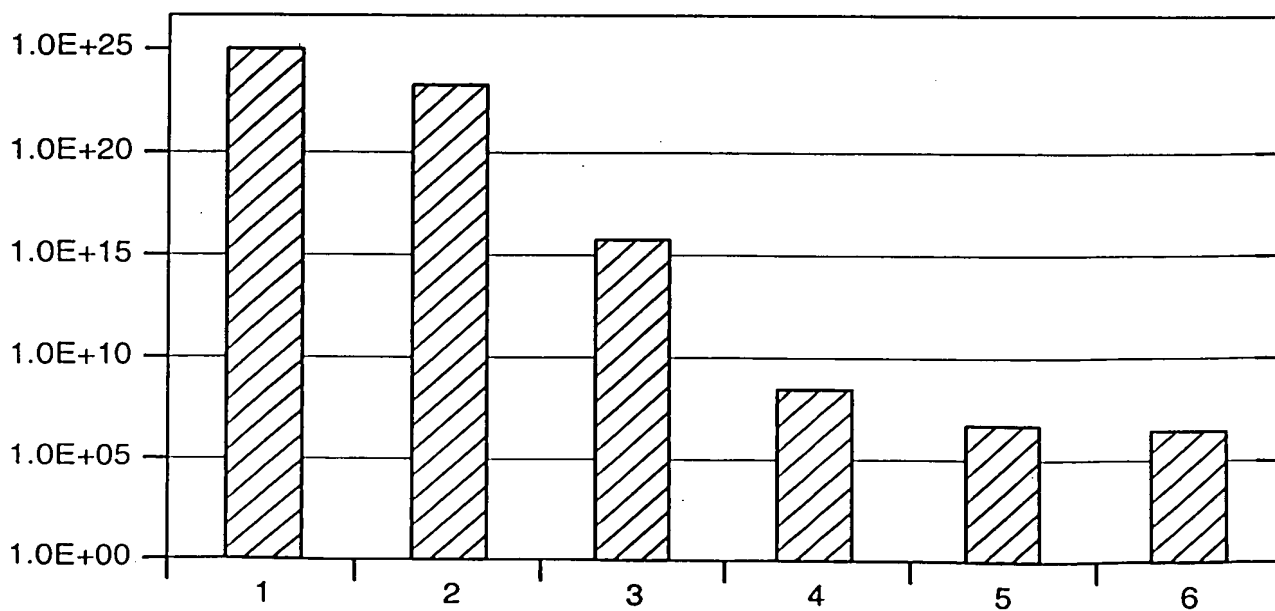


**FIG.\_1**



**FIG.\_2**

4004200 4004200

09782004.021201  
102120.4002860



**FIG. 3**

09782604 021201  
T02Y20" 40028Z60

+

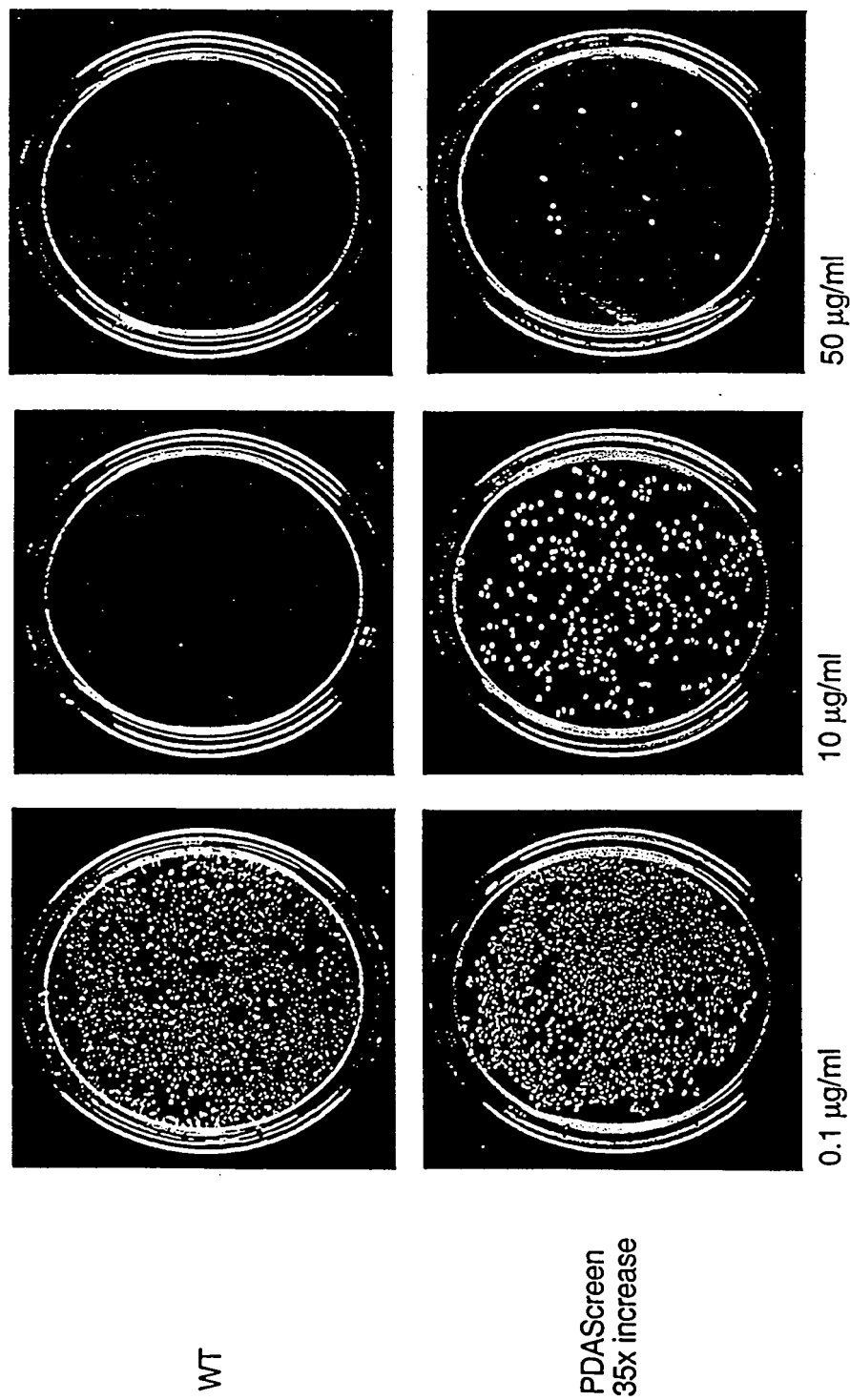
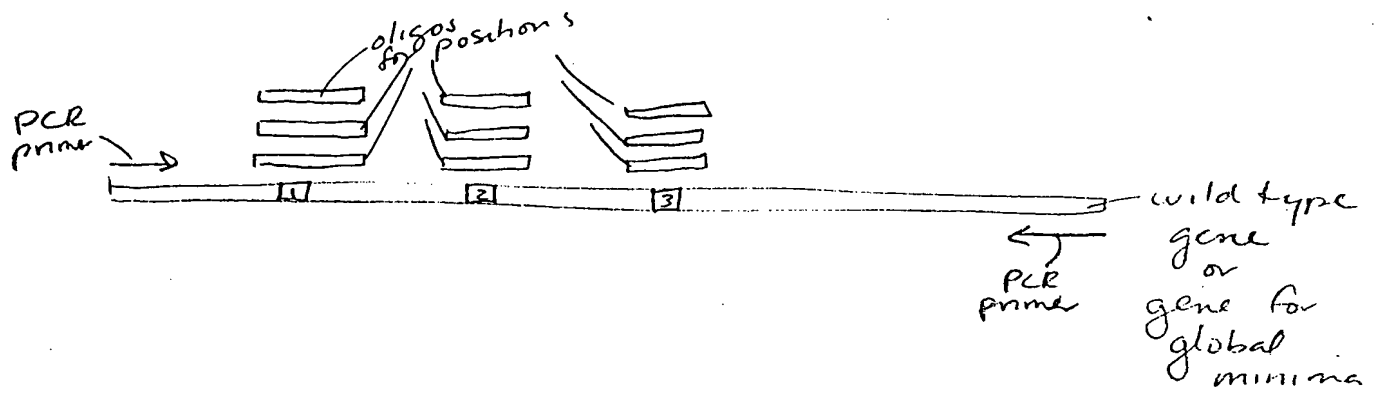


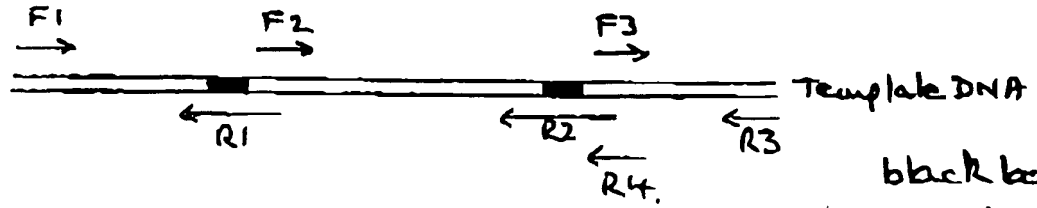
FIG. 4

Figure 5



05783004 034301  
T00T90 40088450

# DIAGRAM

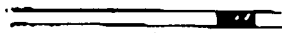


black box  
= region to  
be mutated.

Step 1: Setup 3 PCR reactions.

Products:

Tube 1:



Tube 2:

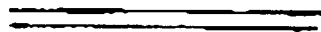
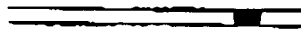


Tube 3:

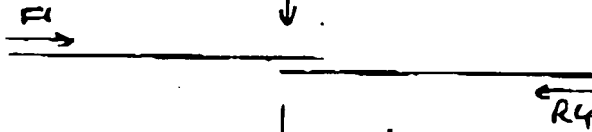


Fig 6A

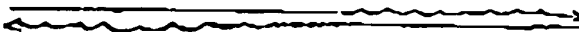
Step 2: Set up PCR reaction with products of tube 1  
+ products tube 2 + F1 + R4



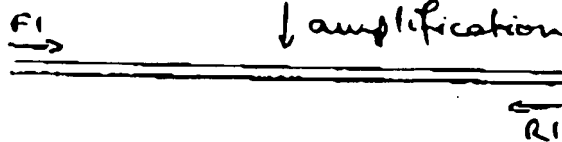
↓ Heat + anneal phase of PCR,



↓ synthesis phase of PCR.



↓ amplification phase, using F1 + R4.  
during subsequent cycles.



Step 3

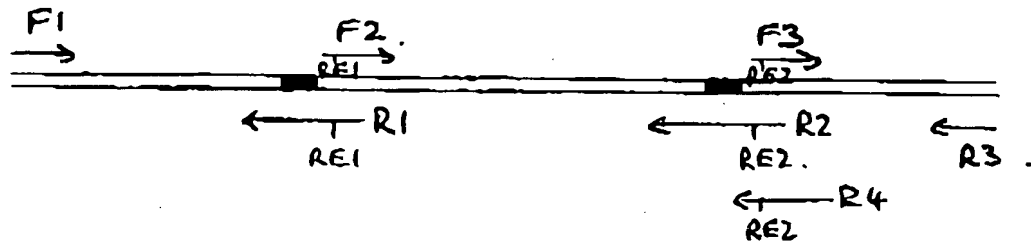
Repeat step 2 using product from step 2 +  
product from Step 1, tube 3 + primers F1 or R3.

Fig 6B

03782004 021204  
T02T20 40020650

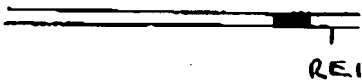
## DIAGRAM 2

Fig 7A

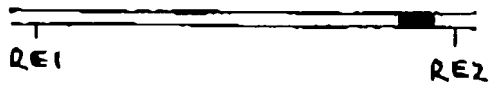


Step 1 Set up 3 PCR reactions:

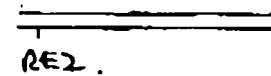
Tube 1:



Tube 2:



Tube 3:



Step 2: digest products from Step 1 with suitable restriction endonucleases

Step 3: ligate digested product from Step 2, Tube 2 with digested product from Step 2, Tube 1.



Step 4

Amplify <sup>via PCR</sup> ligated products of Step 3 with F1 + R4.

Step 5

Digest amplified product of step 4 with restriction endonuclease #2.

Step 6

Ligate product from Step 5 with product from Step 2, tube 1.

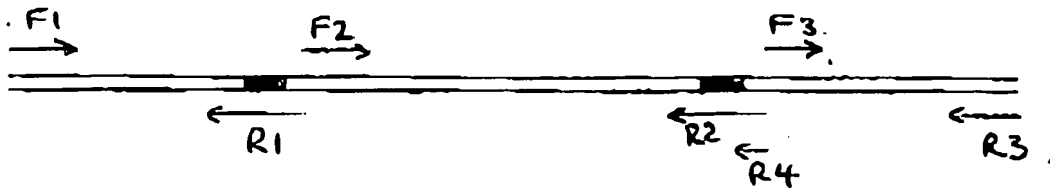
Step 7

Amplify product from step 6 with F1 + R3

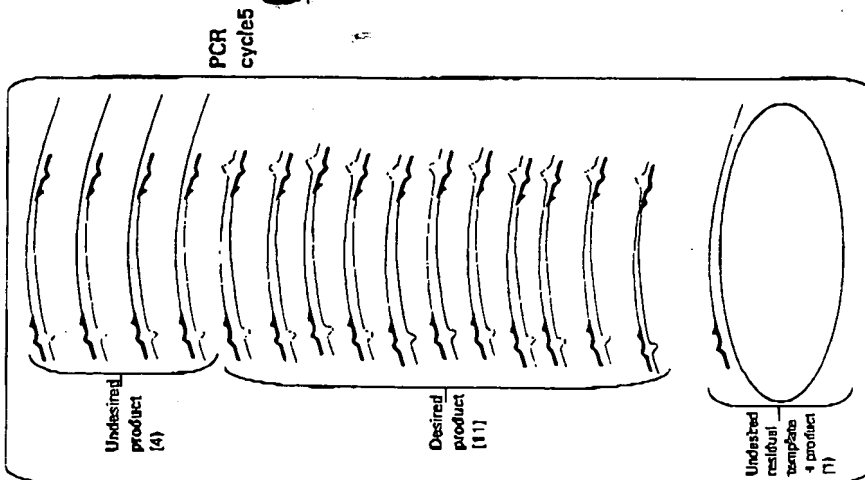
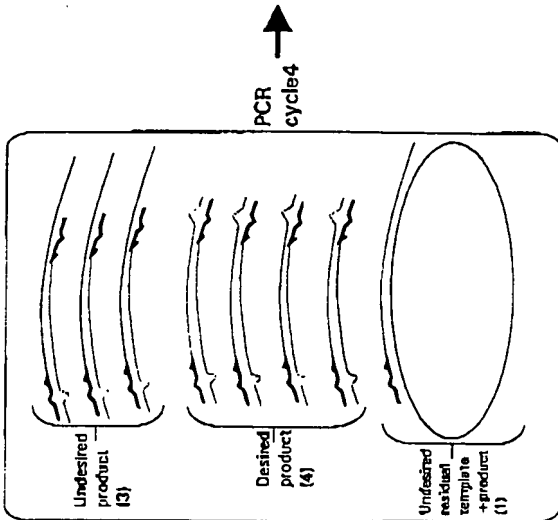
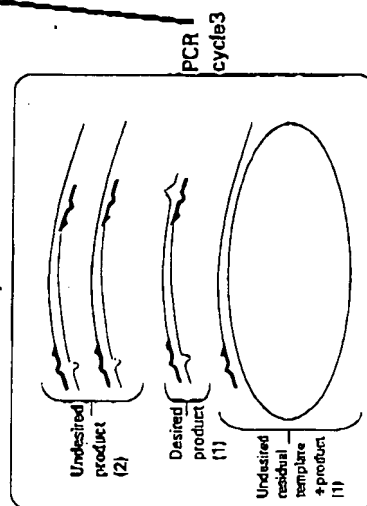
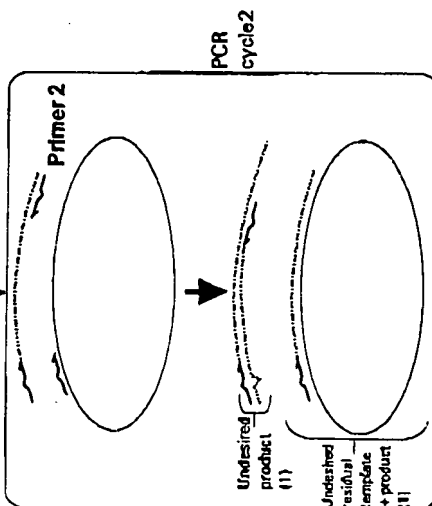
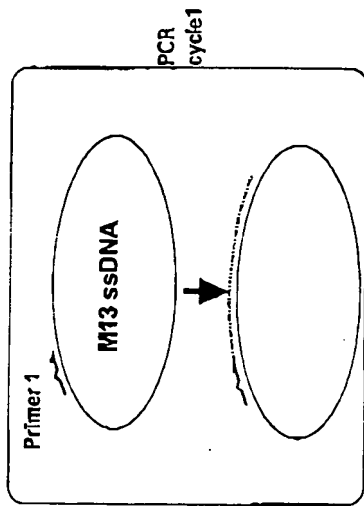


Diagram 3

Fig 8



# Amplification Scheme Based on M13 Single Stranded Template



Numerical progression of desired product with increasing PCR cycles

PCR cycles	Desired Product	Undesired Products and residual template	Percent Desired Product in Total Product
1	1	1	0.00%
2	1	1	0.00%
3	1	1	25.00%
4	4	4	50.00%
5	11	5	68.75%
6	28	6	81.25%
7	57	7	89.06%
8	120	8	93.75%
9	247	9	96.48%
10	502	10	98.05%
11	1013	11	98.93%
12	2036	12	99.41%
13	4083	13	99.68%
14	8178	14	99.83%
15	16369	15	99.91%
16	32752	16	99.95%
17	65519	17	99.97%
18	131054	18	99.99%
19	262125	19	99.99%
20	524258	20	100.00%

Figure 9